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*Total number of authors:*
14

*Published in:*
P L o S One

*Link to article, DOI:*
10.1371/journal.pone.0133526

*Publication date:*
2015

*Document Version*
Publisher's PDF, also known as Version of record

PLOS ONE

RESEARCH ARTICLE

# Aerobic Microbial Respiration In Oceanic Oxygen Minimum Zones

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**Citation:** Kalvelage T, Lavik G, Jensen MM, Revsbech NP, Löscher C, Schunck H, et al. (2015) Aerobic Microbial Respiration In Oceanic Oxygen Minimum Zones. PLoS ONE 10(7): e0133526. doi:10.1371/journal.pone.0133526

**Editor:** Zhe-Xue Quan, Fudan University, CHINA

**Received:** March 30, 2015

**Accepted:** June 29, 2015

**Published:** July 20, 2015



**Data Availability Statement:** NCBI Sequence Read Archive submission (SUB910041, SUB910044, SUB910045, and SUB910046) and all other relevant data are within the paper and its Supporting Information files.

**Funding:** The authors were supported by the following funders: TK, GL, CL, HS, DKD, HH, RK, JLR, RS, MIG, MMK: DFG-funded Sonderforschungsbereich 754 "Climate-Biogeochemistry Interactions in the Tropical Ocean" (www.sfb754.de); TK, GL, MMJ, MH, MMMK: Max Planck Society (www.mpg.de); TK: Canada Excellence Research Chair in Ocean Science and Technology (http://www.cerc.gc.ca/chairholders-

## Abstract

Oxygen minimum zones are major sites of fixed nitrogen loss in the ocean. Recent studies have highlighted the importance of anaerobic ammonium oxidation, anammox, in pelagic nitrogen removal. Sources of ammonium for the anammox reaction, however, remain controversial, as heterotrophic denitrification and alternative anaerobic pathways of organic matter remineralization cannot account for the ammonium requirements of reported anammox rates. Here, we explore the significance of microaerobic respiration as a source of ammonium during organic matter degradation in the oxygen-deficient waters off Namibia and Peru. Experiments with additions of double-labelled oxygen revealed high aerobic activity in the upper OMZs, likely controlled by surface organic matter export. Consistently observed oxygen consumption in samples retrieved throughout the lower OMZs hints at efficient exploitation of vertically and laterally advected, oxygenated waters in this zone by aerobic microorganisms. In accordance, metagenomic and metatranscriptomic analyses identified genes encoding for aerobic terminal oxidases and demonstrated their expression by diverse microbial communities, even in virtually anoxic waters. Our results suggest that microaerobic respiration is a major mode of organic matter remineralization and source of ammonium (~45-100%) in the upper oxygen minimum zones, and reconcile hitherto observed mismatches between ammonium producing and consuming processes therein.

## Introduction

Most of the organic matter in the world's oceans is remineralized via aerobic respiration by heterotrophic microorganisms. Only when oxygen ($O_2$) becomes scarce, microorganisms use

titulaires/wallace-eng.aspx); NPR: European Research Council (http://erc.europa.eu), grant 267233, and Danish Council for Independent Research: Natural Sciences (http://ufm.dk/en/research-and-innovation/councils-and-commissions/the-danish-council-for-independent-research/the-council-1/the-danish-council-for-independent-research-natural-sciences), grants 10-083140 and 272-07-0057. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing Interests:** The authors have declared that no competing interests exist.

thermodynamically less favourable electron acceptors, predominantly nitrate ($NO_3^-$), for the oxidation of organic matter [1]. Large, permanently $O_2$-depleted water masses favouring $NO_3^-$ respiration, so-called oxygen minimum zones (OMZs), are found in association with tropical and subtropical upwelling systems [2]. These regions are characterized by high surface productivity and thus strong $O_2$ depletion via degradation of sinking organic matter at mid-depth, exacerbated by limited $O_2$ replenishment [3]. Nitrate respiration in OMZs accounts for ~20–40% of global oceanic nitrogen (N) loss [4]. The N-deficient OMZ waters (relative to phosphorus) are eventually upwelled and result in largely N-limited surface primary production at low latitudes [5]. Hence, despite a combined volume of only ~1% ($O_2 \leq 20$ μmol $l^{-1}$) of the global ocean [1], OMZs play an important role in regulating phytoplankton nutrient availability, and thus carbon (C) fixation in the oceans.

Traditionally, OMZ N-loss has been attributed to heterotrophic denitrification [6–8], the step-wise reduction of $NO_3^-$ to dinitrogen gas ($N_2$) coupled to the oxidation of organic matter by facultative anaerobes at low $O_2$ tensions [9]. In the last decade, however, numerous studies have identified anammox, the anaerobic oxidation of $NH_4^+$ with nitrite ($NO_2^-$), as a major $N_2$-forming pathway in OMZs, often exceeding N-loss via denitrification [10–14]. Assuming denitrification to be the only N-remineralization pathway in OMZs, anammox activity therein should be constrained by the amount of $NH_4^+$ released during heterotrophic denitrification [15,16]. The apparent decoupling of the two processes requires sources of $NH_4^+$ other than denitrification. Alternative anaerobic $NH_4^+$-producing pathways in OMZs include heterotrophic $NO_3^-$ reduction to $NO_2^-$, dissimilatory $NO_3^-$ reduction to $NH_4^+$ (DNRA) and sulphate reduction coupled to organic matter degradation [13,17–19]; yet, rates reported for these processes are neither sufficient to fully account for estimated remineralization of sinking organic matter in OMZs [14] nor to explain the $NH_4^+$ demands of concurrent anammox activity. Particularly large imbalances between $NH_4^+$ sources and sinks persist at the upper OMZ boundaries, where rates of anammox as well as aerobic $NH_4^+$ oxidation often peak [14,17,20,21].

Microaerobic respiration of organic matter has been suggested to provide the "missing" $NH_4^+$ in the upper OMZs [14,17]. Owing to the detection limit of conventional $O_2$ measurements, remineralization of organic matter below ~5 μmol $l^{-1}$ of $O_2$ is commonly believed to proceed via $NO_3^-$ respiration [22]. However, during a recent hydrochemical survey of the South Pacific OMZ with switchable trace amount oxygen (STOX) sensors, accumulations of $NO_2^-$, considered a proxy for active $NO_3^-$ respiration, were only observed at $O_2$ levels <50 nmol $l^{-1}$ [23]. At the same time, $O_2$-dependent nitrification at $O_2$ levels ≤1 μmol $l^{-1}$ in OMZs hints at aerobic microbial respiration to be well adapted to nanomolar $O_2$ concentrations [14,24,25]. In accordance, highly sensitive measurements of (microbial) $O_2$ consumption in the OMZs off Chile and Mexico recently revealed apparent half-saturation coefficients ($K_m$) for aerobic respiration of ~10–200 nmol $O_2$ $l^{-1}$ [26].

Highly efficient $O_2$ scavenging is a prerequisite for maintaining anoxic conditions in OMZs against the transport of $O_2$-bearing water masses via turbulent mixing, local downwelling or lateral advection. Fundamentally, the balance between microbial $O_2$ uptake and downward $O_2$ transport via turbulent diffusion results in a gradual decrease of $O_2$ and the typical oxycline formation. In such a simplistic setting, sub-oxycline waters quickly become functionally anoxic [23]. Intrusions, local downwelling and lateral advection of oxygenated water masses, however, provide effective means of $O_2$ transport into the OMZ interior [27,28]. To maintain anoxia in OMZs, such $O_2$ injections must either be rare events or well-adapted, opportunistic microbial communities must rapidly draw down any $O_2$ available.

Here, we assessed the potential of microaerobic respiration and the importance of aerobic organic matter degradation as a source of $NH_4^+$ in the OMZs off Namibia and Peru, using an $^{18\text{-}18}O_2$ labelling approach suitable for $O_2$ consumption measurements at low $O_2$

concentrations [29]. Rate measurements were complemented by analyses of metagenomes and metatranscriptomes from the South Pacific OMZ, for presence and expression of key-functional genes involved in aerobic respiration. Further, we explored the effects of $O_2$ depletion associated with marine snow particles on microbial respiration, by combining $^{18-18}O_2$ labelling experiments with *in-situ* particle size analysis and modelling of aggregate-size-dependent respiration.

## Materials and Methods

### Water sampling and physico-chemical measurements

Samples were taken on cruises M76-2 and M77-3 over the Namibian shelf (May to June 2008) and in the OMZ off Peru (December 2008 to January 2009), respectively, on board R/V Meteor (Table 1). Seawater was collected with either a conductivity-temperature-depth (CTD) rosette system fitted with 10-L Niskin bottles or a pump-CTD system (depth range: ~375 m). Off Namibia, a custom-built bottom water sampler [30] was used to collect additional samples from the benthic boundary layer (BBL). Oxygen was measured with a conventional amperometric microsensor and a CTD-mounted switchable trace amount oxygen (STOX) sensor [31] (detection limit: 50–100 nmol $l^{-1}$) for high-accuracy $O_2$ measurements at selected depths. Continuous vertical profiles of chlorophyll *a* were obtained fluorometrically and calibrated against discrete values derived from acetone extraction. Ammonium concentrations were determined fluorometrically [32] on discrete high-resolution samples (1–2 m). Samples for particulate organic nitrogen (PON) were filtered onto pre-combusted GF/F filters (Whatman), stored frozen, and measured on an elemental analyzer (EURO EA and Thermo Flash EA, 1112 Series) after drying and decalcification with fuming hydrochloric acid.

Particle abundances and size distributions were measured during cruise M93 (February to March 2013) to the central Peruvian OMZ (Table 1), using an Underwater Vision Profiler (UVP5) [33]. A total of 138 UVP5 profiles, quantifying particles with an equivalent spherical diameter (ESD) of 0.06–26.8 mm, were obtained.

### Determination of $O_2$ consumption rates

Microaerobic respiration in the Namibian as well as the coastal and offshore Peruvian OMZ was measured as the consumption of $^{18-18}O_2$ in time-series incubations. At each station, up to six depths were chosen for $^{18-18}O_2$ labelling experiments (S1 Table). A detailed description of the experimental procedure is given in reference [29]. Briefly, Helium-purged water samples were adjusted to the following $^{18-18}O_2$ concentrations by adding a defined volume of sterile-filtered seawater containing ~1 mmol $^{18-18}O_2$ $l^{-1}$ (Sigma-Aldrich, Germany): ~2.5 μmol $l^{-1}$ (Namibian OMZ), ~2.5–7.5 μmol $l^{-1}$ (upper Peruvian OMZ) and ~1 μmol $l^{-1}$ (Peruvian OMZ core). At selected stations, additional $O_2$ sensitivity assays were carried out, in which replicate samples were adjusted to different $^{18-18}O_2$ concentrations in the range of ~0.5–20 μmol $l^{-1}$ (S2 Table). Following $O_2$ adjustment, subsamples were filled into 12-ml Exetainers (Labco, UK), using Helium overpressure to avoid $^{16-16}O_2$ contaminations. One Exetainer each was sacrificed to determine initial total $O_2$ ($^{18-18}O_2$ + $^{16-16}O_2$) concentrations with a fast-responding Clark-type $O_2$ microsensor (MPI Bremen; detection limit: ~0.5 μmol $l^{-1}$). Samples were incubated in the dark at mean *in-situ* temperatures and duplicates were inactivated after ~0, 3, 6, 12, 24 and 48 h by adding saturated mercuric chloride. Final $^{18-18}O_2$ concentrations were determined using membrane inlet mass spectrometry (MIMS; GAM200, IPI) in a shore-based laboratory. A two-point calibration was performed based on the $^{16-16}O_2$ reading for air-saturated water pumped across the inlet membrane and the $^{18-18}O_2$-background signal with the pump turned off. Oxygen consumption rates were calculated from the slope of linear regression of $^{18-18}O_2$

**Table 1. Overview of sampling locations and times for the various types of data considered in this study.**

| Cruise | OMZ | Lat/Lon | Year | Season | Data obtained |
|---|---|---|---|---|---|
| Meteor 76–2 | Namibia | 19–23°S/12-14°E | 2008 | Austral autumn | $O_2$ consumption rates, N-cycling rates |
| MOOMZ-1 [34] | Chile | 20°07'S/70°23'W | 2008 | Austral winter | Terminal respiratory oxidase gene and transcript abundance |
| Meteor 77–3 | Peru | 4–16°S/75-84°W | 2008–2009 | Austral summer | $O_2$ consumption rates, N-cycling rates, terminal respiratory oxidase gene abundance |
| Meteor 93 | Peru | 12–14°S /76-79°W | 2013 | Austral summer | *In-situ* particle size spectra |

doi:10.1371/journal.pone.0133526.t001

concentrations as a function of time and corrected for $^{16-16}O_2$ background concentrations. Samples incubated for ~48 h were not considered if a non-linear decrease of $^{18-18}O_2$ was observed for the final incubation period (24–48 h).

### Modelling of $O_2$ consumption rates

The diffusive $O_2$ flux at the upper OMZ boundary was calculated from turbulent diffusivity and the $O_2$ concentration gradient according to Fick's law. Oxygen consumption rates were then estimated from $O_2$ flux gradients. A more detailed description of the modelling approach is given in the S1 File.

### Metagenomic and metatranscriptomic analyses

In the Peruvian OMZ, large-volume samples (>300 L) for nucleic acid extraction were collected onto 0.22-μm Durapore Membrane filters (Millipore), after passing an 8-μm pre-filter, using *in-situ* pumps (WTS-LV, McLane). Upon recovery, filters were immediately frozen and stored at -80°C until further analysis. DNA was extracted using a chloroform-phenol extraction protocol [35]. Sequencing of 2.5-μg DNA samples was done on a GS-FLX 454 pyrosequencer (Roche). A detailed description of the raw read processing as well as the functional (cytochrome oxidase type) and taxonomic sequence assignment is given in the S1 File.

Metagenomic and metatranscriptomic data from the Chilean OMZ (<1.6 μm fraction; Table 1) were provided by F. Stewart and E. DeLong. For further details on sample collection, sequencing and sequence post-processing please refer to reference [34]. Here, BLAST hits for all non-replicate, non-rRNA sequences (DNA and cDNA) were analysed for gene abundance and expression of the various types of cytochrome oxidases and their taxonomic assignments.

## Results and Discussion

### Oxygen distributions

The stations investigated on the Namibian shelf (19°S-23°S) were characterized by high surface chlorophyll *a* concentrations, i.e. high primary productivity, at the time of sampling [25]. Oxygen concentrations in the surface waters ranged from ~150 to 250 μmol $l^{-1}$ and gradually declined to ≤15 μmol $l^{-1}$ (here used as a cut-off for the upper OMZ boundary) at ~65–85 m depth (Fig 1a and S1 Fig). At two sampling sites (station 225 and 252), steep $O_2$ gradients in the upper OMZ as well as non-detectable levels of $O_2$ (≤100 nmol $l^{-1}$) by STOX sensor measurements in the lower OMZ (S1 Table), indicated apparently anoxic conditions over the shelf. In contrast, $O_2$ concentrations in the lower micromolar range (~2–6 μmol $l^{-1}$) persisted throughout the OMZ at nearby stations (231 and 243). Both $O_2$ and density gradients indicated

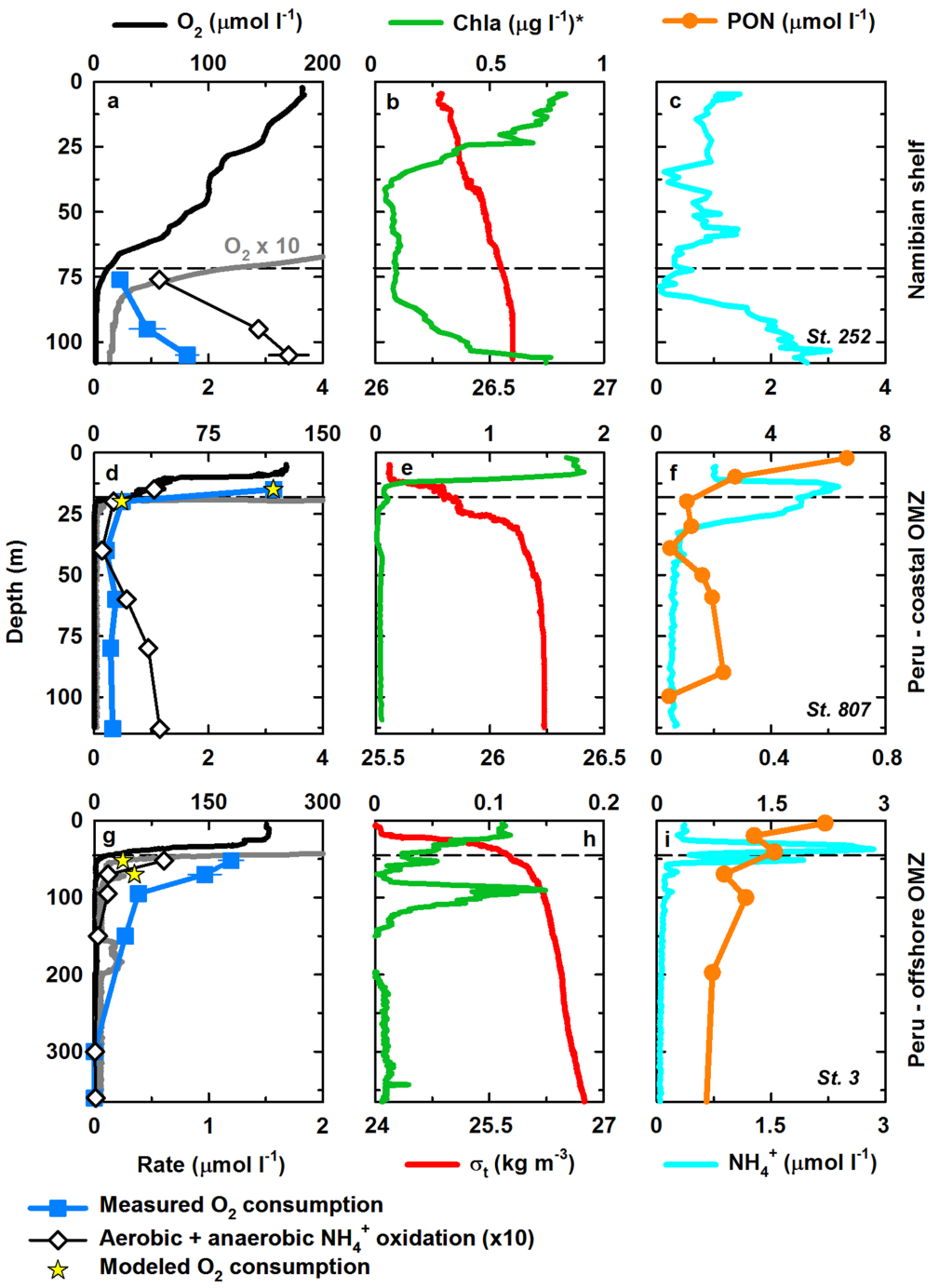


**Fig 1. Physicochemical zonation and rates of microbial respiration in the OMZs off Namibia and Peru.** (a-c) Namibian shelf (station 252, 111m). (d-f) Peruvian coastal OMZ (station 807, 115 m). (g-i) Offshore Peruvian OMZ (station 3, 4697 m). Dashed lines indicate the upper OMZ boundary ($O_2 \leq 15$ µmol $l^{-1}$). Previously determined rates of aerobic and anaerobic $NH_4^+$ oxidation [14,24,25] are tenfold magnified. Please note the differences in scale between stations. *Chlorophyll *a* concentrations in panel b in relative units.

doi:10.1371/journal.pone.0133526.g001

a rather weak stratification of the Namibian shelf waters, facilitating vertical mixing of more oxygenated surface waters into the OMZ.

In the OMZ off Peru (6°S-16°S), stations covered a wider range of productivity regimes [14]. Sampling sites ranged from highly productive, shallow coastal stations to more oligotrophic, deep offshore ones, as indicated by more than tenfold different chlorophyll *a* levels between stations (Fig 1e and 1h, S1 Fig). Off the central Peruvian coast, surface water $O_2$ concentrations were as low as ~125 μmol $l^{-1}$ (station 807) and rapidly declined to ≤15 μmol $l^{-1}$ at ~20–30 m depth. Further offshore, the oxic mixed layer was more extensive, with the upper OMZ boundary typically located at ~50–70 m depth. Non-detectable $O_2$ levels in the lower OMZ by STOX sensor measurements (≤50 nmol $l^{-1}$; S1 Table) and cross-calibration of the data obtained by conventional $O_2$ sensors with the STOX sensor data, indicated the onset of anoxia ~20–50 m below the OMZ boundary. In agreement, extensive STOX measurements during a recent hydrochemical survey of the South Pacific OMZ showed these waters to be $O_2$-depleted down to at least 10 nmol $l^{-1}$ [23]. Generally pronounced pycnoclines indicated a stronger vertical stratification off Peru, as compared to the Namibian shelf OMZ, and thus reduced mixing across the upper OMZ boundary. Further offshore, episodic lateral advection of $O_2$-enriched waters into the lower OMZ was however clearly evident from local $O_2$ maxima (5–25 μmol $l^{-1}$) at 50–100 m below the oxycline (station 3 and 36; Fig 1g and S1 Fig).

## Oxygen consumption rates

On the Namibian shelf, aerobic respiration was measured at four sampling sites in $^{18\text{-}18}O_2$ labelling experiments, from the upper OMZ boundary down to the sediment-water interface (S1 Table). Oxygen consumption rates were fairly consistent between stations and depths, typically ranging from ~0.15 to 0.5 μmol $O_2$ $l^{-1}$ $d^{-1}$, and showed no significant correlation with *in-situ* $O_2$ concentrations (Spearman $p > 0.05$). The latter may, at least in part, owe to the uniform $^{18\text{-}18}O_2$ adjustments (~2.5 μmol $l^{-1}$) of the incubations irrespective of *in-situ* $O_2$ levels, thus stimulating or inhibiting aerobic respiration compared to *in-situ* activity. Noticeably higher rates (~1–1.6 μmol $O_2$ $l^{-1}$ $d^{-1}$) were observed at one station (252) in the lower OMZ and the shelf bottom waters. In this zone, enhanced potential for aerobic respiration likely resulted from a high availability of fresh organic matter, as indicated by high chlorophyll *a* levels in the lower OMZ (Fig 1b).

Off Peru, $^{18\text{-}18}O_2$ labelling experiments were carried out at seven stations, with $^{18\text{-}18}O_2$ adjustments aimed at mimicking the $O_2$ gradient from the upper OMZ boundary towards the OMZ core. Here, the incubations revealed a significant correlation between $O_2$ consumption rates and *in-situ* $O_2$ concentrations (Spearman $R = 0.76$, $p \leq 0.001$), i.e. high aerobic respiration at the upper OMZ boundary and rapidly decreasing rates towards the OMZ core (Fig 1d and 1g, S1 Fig). Maximum $O_2$ consumption rates in the upper OMZ waters were >3 μmol $O_2$ $l^{-1}$ $d^{-1}$ near the Peruvian coast and declined to ~1 μmol $O_2$ $l^{-1}$ $d^{-1}$ at the open ocean stations (S1 Table), consistent with observed shelf-offshore gradients in export production for the region [14]. In the lower (anoxic) Peruvian OMZ, potential rates of aerobic respiration showed little variability within and between stations, and typically ranged from ~0.2 to 0.4 μmol $O_2$ $l^{-1}$ $d^{-1}$. At the stations furthest offshore, i.e. the least productive ones (station 3 and 5), $O_2$ consumption in the core of the OMZ was below the detection limit of the $^{18\text{-}18}O_2$ labelling approach employed here (~0.1 μmol $O_2$ $l^{-1}$ $d^{-1}$).

Given the methodological challenges of accurately determining $O_2$ concentrations in the lower micromolar range [29,31], very few direct measurements of aerobic respiration in OMZs exist for comparison. Oxygen consumption rates of similar magnitude (~0.5–2 μmol $O_2$ $l^{-1}$ $d^{-1}$), have recently been measured using STOX sensors in low-$O_2$ waters of the productive North

and South Pacific coastal OMZs [26,31]. Earlier estimates of aerobic respiration in the Eastern Pacific upwelling regions based on particle flux attenuations [36] also compare well with upper OMZ respiration rates determined in this study. Calculating $O_2$ flux gradients provides another indirect approach to estimate $O_2$ consumption rates. Here, respiration rates were modelled for incubation depths near the upper OMZ boundary at selected stations off Peru (Fig 1d and 1g, S1 Fig). Modelled rates matched measured ones (±5%) at two sites (station 807 and station 36 at 90 m), but were significantly lower (~65–90%) for the remaining sampling locations. Higher experimentally determined $O_2$ consumption rates may partially owe to a stimulation of aerobic respiration by slightly elevated $^{18\text{-}18}O_2$ levels in the incubations compared to $O_2$ concentrations *in-situ*. On the other hand, lower modelled respiration rates are expected since the diffusion-controlled 1-D model implies steady state conditions, and balances the rates with the vertical diffusive transport only. Any additional advective $O_2$ transport and related transient effects are neglected, resulting in either similar or lower modelled respiration rates compared to measured ones. Indeed, local sub-oxycline $O_2$ maxima can be observed at several stations (Fig 1g and S1 Fig), the majority of which are likely associated with advective $O_2$ transport. In some instances, locally elevated $O_2$ concentrations might indicate photosynthetic $O_2$ production within the OMZ (Fig 1g and 1h) [26].

Overall, the lateral distribution of aerobic respiration at the upper OMZ boundaries, i.e. at micromolar $O_2$ levels, appears to be largely controlled by the availability of organic matter, as indicated by maximum $O_2$ consumption rates in organic-rich coastal waters (Fig 1f and 1i) [14,29]. Less variable, but significant potential for aerobic respiration was also consistently measurable in samples from apparently anoxic depths, particularly in the core of the Peruvian OMZ. Here, actual rates of microbial $O_2$ consumption can be expected to primarily depend on the presence or absence of trace $O_2$ concentrations. A recent study concluded that the South Pacific OMZ core is largely functionally anoxic and possibly only contains picomolar concentrations of $O_2$, due to efficient $O_2$ scavenging by microorganisms [23]. At the same time, however, the traditional view of largely static $O_2$-deficient zones increasingly shifts towards temporally more dynamic OMZs. Large-scale observations indicate episodic $O_2$ injections into the Peruvian OMZ via intrusions of oxygenated surface waters or mixing events, such as related to eddy activity [27,28]. Further, $O_2$-bearing water layers surrounded by hundreds of meters of anoxic waters indicate lateral $O_2$ supply into the OMZs (Fig 1g) [26]. These pulses of $O_2$ can, at least temporarily, sustain aerobic respiration in otherwise anoxic waters. The total flux of $O_2$ via episodic $O_2$ injections is difficult to assess since the frequency of observations of sub-oxycline $O_2$ maxima is likely reduced due to dispersion and efficient microbial $O_2$ consumption. However, the importance of such transient $O_2$ transport is indicated by recent biogeochemical modelling of the South Pacific OMZ, which hints at substantial aerobic organic matter degradation in the OMZ, and lateral advection as an important source of $O_2$ for aerobic respiration therein [37].

## Aerobic terminal oxidases gene abundance and expression

Identification of cytochrome oxidases, catalysing the terminal electron transfer during oxic respiration, in the South Pacific OMZ provided further evidence for active aerobic respiration at near-anoxic levels of $O_2$. Metagenomes obtained in the Peruvian offshore OMZ (station 3) as well as metagenomes and metatranscriptomes previously collected off Chile [34], revealed presence and expression of genes encoding for low-affinity cytochrome *c* oxidases ($K_m$ ~200 nmol $O_2$ $l^{-1}$) as well as high-affinity cytochrome *bd* and *cbb*$_3$ oxidases ($K_m$ <10 nmol $O_2$ $l^{-1}$) [38] from the oxycline down to the OMZ core (Fig 2 and S3 Table).

In all samples investigated, the majority of aerobic oxidase genes and gene transcripts were of the common low-affinity type (68–99%). Cytochrome *c* oxidases could largely be assigned to

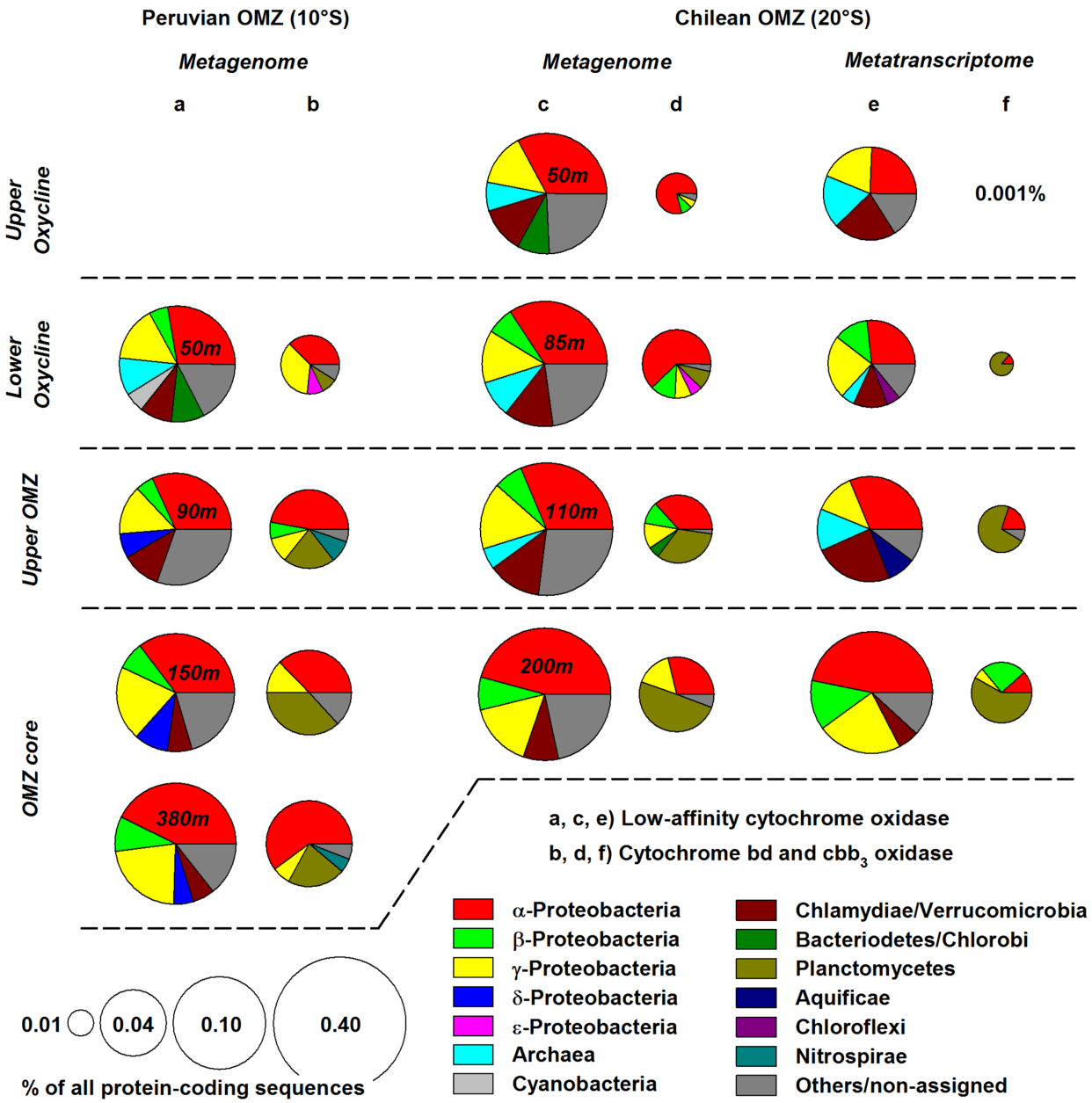


**Fig 2. Abundance of genes and transcripts encoding for terminal respiratory oxidases in the ETSP OMZ.** (a, b) Abundance of low-affinity (cytochrome c oxidase) and high-affinity (cytochrome bd and $cbb_3$ oxidase) aerobic oxidases in the Peruvian OMZ (station 3). (c-f) Abundance and expression of cytochrome oxidase genes in the OMZ off Chile during cruise MOOMZ-1 [34]. Taxonomic affiliations of cytochrome oxidases are shown on domain, phylum or class level if represented by at least 5% of oxidase-coding sequences. Exact abundance and expression levels as well as taxonomic assignments of the individual types of cytochrome oxidases are given in S3 Table.

doi:10.1371/journal.pone.0133526.g002

the phylum of Proteobacteria (mostly Alpha-, Beta- and Gammaproteobacteria), which is generally dominant in OMZs [39], followed by the Chlamydiae-Verrucomicrobia group. Consistent with a prior study [34], one of the most abundant taxa was the heterotrophic marine genus *Pelagibacter* (Alphaproteobacteria), accounting for up to 12% and 15% of all respiratory oxidase genes and gene transcripts, respectively, in the Chilean OMZ oxycline. In general, heterotrophs appeared to dominate the low-affinity type microbial community, yet also a number of

cytochrome *c* oxidases strongly similar to those of marine chemo- and photoautotrophic prokaryotes were identified. Most notably, cytochrome *c* oxidases of the archaeal $NH_4^+$ oxidizer *Nitrosopumilus maritimus* were highly abundant and expressed (2–5% and 4–15% of all aerobic oxidase DNA and RNA sequences, respectively) in the oxycline and upper OMZ off Peru and Chile. In this zone, *N. maritimus* abundances, $NH_4^+$-oxidizing activity as well as archaeal ammonia monooxygenase subunit A gene and transcript numbers are generally elevated [14,17,34,40,41]. Further, low-affinity oxidase genes and gene transcripts of $NH_4^+$-oxidizing (*Nitrosococcus* and *Nitrosomonas*) and $NO_2^-$-oxidizing bacteria (*Nitrobacter*, *Nitrococcus and Nitrospira*) were detected throughout the OMZ, in line with active bacterial nitrification in the South Pacific [14,17,42] and other oceanic OMZs [25,43,44]. In addition, DNA and RNA sequences collected in the Peruvian and Chilean OMZ, respectively, matched cyanobacterial cytochrome *c* oxidases, mostly of the genus *Prochlorococcus*; off Peru, noticeably high abundances (1–5% of all aerobic oxidase DNA sequences) coincided with the deep chlorophyll *a* maximum (Fig 1h) as well as previously identified *Prochlorococcus*-specific marker pigments [45] at this station. These low-light-adapted cyanobacteria are widely distributed across the major OMZs [46,47], and likely also provide a local source of $O_2$ via oxygenic photosynthesis well below the oxycline [26].

A large variety of aerobic and anaerobic microorganisms have adapted to microoxic environments by evolving terminal respiratory oxidases with high $O_2$ affinities [38]. For example, the cytochrome *bd* oxidase of *Escherichia coli* with an apparent $K_m$ value of 3–8 nmol $O_2$ $l^{-1}$ is maximally expressed under microaerobic conditions [48,49], permitting aerobic growth at lower nanomolar, possibly even picomolar $O_2$ concentrations [50]. Another high-affinity respiratory oxidase, the cytochrome $cbb_3$ oxidase, has a similarly low $K_m$ value of 7 nmol $O_2$ $l^{-1}$ [51]. Off the Peruvian and Chilean coasts, DNA and RNA sequences of both types of high-affinity oxidases could mostly be assigned to auto- and heterotrophic alpha-, beta and gammaproteobacterial taxa (e.g. *Rosebacter*, *Ralstonia* and sulphur-oxidizing symbionts, respectively) as well as Planctomycetes (Fig 2 and S3 Table). In the OMZ and lower oxycline, cytochrome *bd* and $cbb_3$ oxidases closely resembling those of the anammox bacterium Candidatus *Kuenenia stuttgartiensis* (Planctomycetes) were present in particularly high abundances (up to 49% and 86% of all high-affinity type DNA and RNA sequences, respectively). Presumably, the $O_2$-sensitive anammox bacteria [52] use high-affinity cytochrome oxidases as a means of detoxification [53], enabling them to remain active in a broader $O_2$ regime [24]. Further, in the Peruvian OMZ metagenomes on average 6% of high-affinity oxidases identified were strongly similar to the cytochrome *bd* oxidase of the $NO_2^-$ oxidizer Candidatus *Nitrospira defluvii*, in line with active $NO_2^-$ oxidation at sub-micromolar $O_2$ levels in the South Pacific OMZ [14,23].

Along the $O_2$ gradients investigated, the relative abundance of aerobic oxidase genes and gene transcripts increased from the oxycline towards the lower OMZ. High-affinity cytochrome oxidases were particularly enriched in (near) anoxic zone samples, on both DNA and RNA level. Off Peru and Chile, the ratio of high-affinity to low-affinity oxidase-coding genes increased from 0.14 (50 m) to 0.31 (90–380 m) and 0.01 (50 m) to 0.12 (85-200m), respectively. Most notably, cytochrome *bd* and $cbb_3$ oxidase transcripts in the Chilean OMZ accounted for only 0.001% of all protein-coding sequences at the upper oxycline (~100 μmol $O_2$ $l^{-1}$) [34], while showing a 30-fold higher relative abundance (0.03%) in the presumably anoxic OMZ core (Fig 2f). A recent metatranscriptomic study of a sulphidic event off central Peru, similarly revealed a distinct trend of increasing cytochrome $cbb_3$ oxidase expression as $O_2$ concentrations decreased [54]. Intriguingly, the ratio of high-affinity to low-affinity oxidase transcripts peaked at the lower oxycline and upper OMZ (0.17; $0 < O_2 \leq 10$ μmol $l^{-1}$). At the more oxygenated upper oxycline and at the OMZ core, where the diffusive $O_2$ flux can be expected to equal zero, high-affinity type fractions were significantly reduced (0.12 and 0.04, respectively).

Enhanced expression of high-affinity oxidases in the microoxic transition zone overlying the $O_2$-depleted OMZ core clearly demonstrates the capacity of microorganisms to exploit sub-micromolar $O_2$ concentrations. In corroboration, apparent $K_m$ values for aerobic respiration of 10–200 nmol $O_2$ $l^{-1}$ have recently been reported for the North and South Pacific OMZs [26,31]. Further, identification of cytochrome oxidase genes and gene transcripts revealed broadly similar microbial community compositions between the Chilean and Peruvian OMZ, suggesting microaerobic respiration to be a universal feature of oceanic OMZs. Hence, although $O_2$ concentrations in OMZs remain largely below detection, $O_2$-dependent nitrification and heterotrophic aerobic respiration still proceed, due to efficient $O_2$ scavenging of microbial communities that are well adapted to these (transiently) microoxic environments.

## Oxygen sensitivity of aerobic respiration

At the upper OMZ boundaries, steep $O_2$ gradients mark the transition from oxic to anoxic environments. Experiments simulating changing $O_2$ concentrations (~0.5–20 μmol $l^{-1}$) in this transition zone, revealed a near-linear decrease of $O_2$ consumptions rates with decreasing levels of $O_2$ in the Namibian and Peruvian OMZ (Fig 3a and S2 Table); a surprising result, given that $K_m$ values of aerobic respiratory oxidases are two to three orders of magnitude lower than ambient $O_2$ concentrations in the incubations. Recent studies targeting aerobic $NH_3$ and $NO_2^-$ oxidation, showed both processes to be also mostly insensitive to decreasing $O_2$ concentrations over the same range [14,24,25]. Assuming $O_2$ consumption in our experiments to be largely coupled to heterotrophic activity, the apparent high $O_2$ sensitivity observed here may result from $O_2$ diffusion limitation of aggregate-associated organic matter respiration [29].

High fluxes of particulate matter [36] and pronounced $O_2$ deficiency in OMZs provide ideal conditions for the development of $O_2$-depleted microenvironments [55,56]. Anoxic micro-niches inside marine snow aggregates have been suggested to exist at environmental $O_2$ concentrations up to several tens of micromoles [57–59], thereby extending the effective anoxic OMZ volume [24]. *In-situ* particle size spectra in the Peruvian OMZ show a large fraction of particles larger than ~0.9 mm (Fig 3b, S2 Fig), above which size-dependent model results indicate diffusion-limited respiration for $O_2$ $\leq$20 μmol $l^{-1}$ (Fig 3a and 3b). Moreover, organic-rich aggregates are hot-spots of microbial activity [60], and large fractions of heterotrophic taxa in OMZs are particle-associated [61,62]. Hence, actual $O_2$ levels encountered by much of the aerobic heterotrophic microbial community may be significantly lower than measured bulk concentrations. Likewise, anaerobic microorganisms that can be found in association with particles in OMZs, such as anammox bacteria [61], may in fact be exposed to $O_2$ concentrations much lower than ambient levels. Indeed, a recent study revealed a tight coupling of aerobic and anaerobic N-cycling processes within cyanobacterial aggregates, and suggests aggregates to be important sites of N-loss at low ambient $O_2$ [59]. Oxygen-reduced micro-niches might explain the observed apparent low $O_2$ affinity and high $O_2$ tolerance (>>1 μmol $l^{-1}$) of aerobic and anaerobic microorganisms in OMZs, respectively, under stagnant experimental conditions [24]. Their true $K_m$ values for $O_2$ as well as $O_2$ sensitivities are more likely in the nanomolar range, i.e. closer to those reported from culture studies and stirred environmental samples, in which microbial $O_2$ exposure equals ambient $O_2$ levels [26,38,50,52,63].

## Ammonium release by microaerobic organic matter respiration

Recent reports from major oceanic OMZs have shown that rates of both aerobic and anaerobic $NH_4^+$ oxidation (anammox) often peak near the upper OMZ boundary [13,14,17]. At the same time, anaerobic sources of $NH_4^+$ are generally insufficient to fully explain ammonium oxidation rates in this zone. Particularly denitrification, traditionally regarded as the major N-

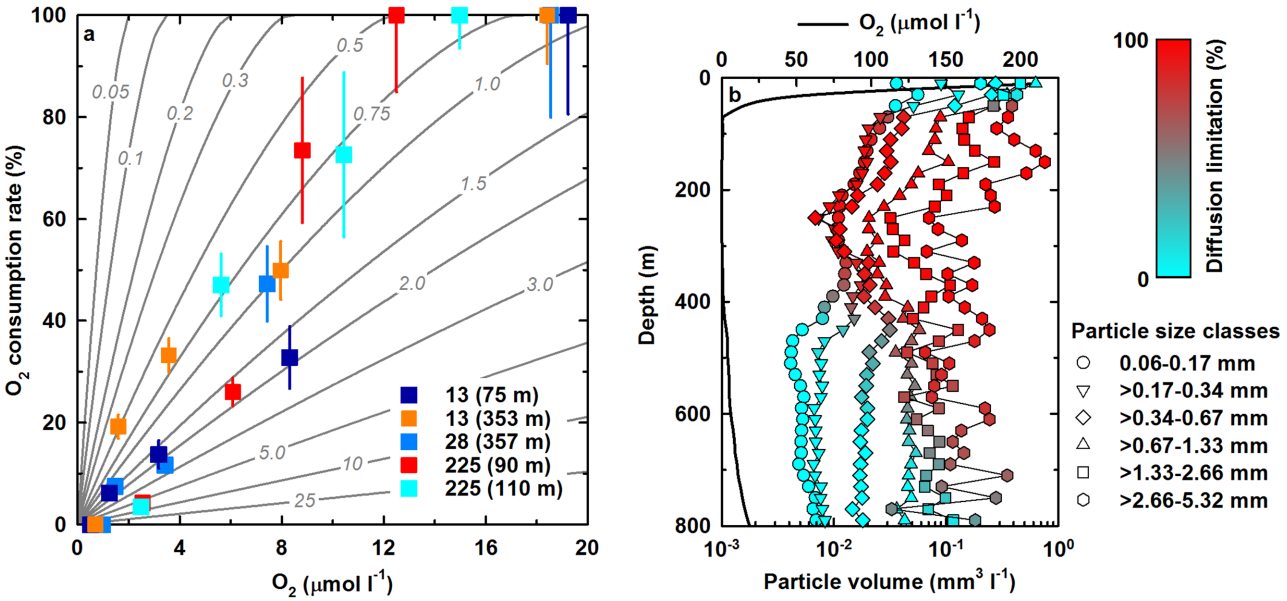


**Fig 3. Oxygen sensitivity of aerobic respiration and OMZ particle size distributions.** (a) $O_2$ sensitivity assays in the Namibian (station 225) and Peruvian OMZ (stations 13 and 28) during cruises M76 and M77-3, respectively. Oxygen consumption rates are given as percentages of the highest rate observed (= 100%) among all $O_2$ treatments (see S2 Table for absolute rates). Error bars for $O_2$ consumption rates are standard errors calculated from linear regression. Isolines (grey) indicate diffusion-limited respiration rates inside aggregates of 0.01–25 mm in diameter. A detailed description of how aggregate-size-dependent rates were calculated is included in the S1 File. (b) Vertical distribution of particle volumes (20 m bins) for six size classes between 0.06 and 5.32 mm (ESD) in the central Peruvian OMZ (12.62°S/77.55°W) during cruise M93. Color shading indicates diffusion limitation of aerobic respiration inside particles. For clarity, particles >5.32 mm are not depicted here. A more general overview of particle size distributions in the ETSP OMZ is given in S2 Fig.

doi:10.1371/journal.pone.0133526.g003

remineralization pathway in OMZs, remained largely undetectable in these studies. In light of so-far unidentified sources of $NH_4^+$, the overall significance of anammox in OMZ N-loss has been questioned [64]. Instead, spatial variability in organic matter fluxes as well as in organic matter stoichiometry have been suggested to result in patchy and thus often missed denitrifying activity [16,65]. We compared the $O_2$ consumption rates determined in this study with previously reported N-cycling rates for the same sampling sites [14,24,25]. In contrast to the proposed large-scale constraint of anammox activity by N-release via denitrification [16], our data suggest ammonium oxidation in the upper OMZs to be largely coupled to microaerobic organic matter remineralization.

Enhanced $O_2$ consumption in the upper Peruvian OMZ coincided with a marked decrease in PON and concomitantly high $NH_4^+$ concentrations (~0.5–2 µmol $l^{-1}$) in this zone (Fig 1f and 1i, S1 Fig), clearly indicating organic matter remineralization via aerobic respiration. For both the upper Peruvian and Namibian OMZ, on average 80% of measured $O_2$ consumption were estimated to be due to the activity of heterotrophic microorganisms, when taking into account $O_2$ consumption via aerobic $NH_4^+$ and $NO_2^-$ oxidation (Table 2). Compared to anaerobic remineralization pathways in this zone, these heterotrophic $O_2$ consumption rates accounted for ~45–100% of organic matter degradation (assuming Redfield stoichiometry: C/N = 6.6), with the remainder mainly attributable to $NO_3^-$ reduction to $NO_2^-$. Further, in waters off both Namibia and Peru rates of heterotrophic oxic respiration, and thus aerobic $NH_4^+$ release, were significantly correlated to rates of total (aerobic + anaerobic) $NH_4^+$ oxidation (Spearman R = 0.69 and 0.50, respectively, $p < 0.01$), emphasizing the tight coupling of $NH_4^+$ producing and consuming processes in OMZs (Fig 1a, 1d and 1g, S1 Fig). Near the upper OMZ boundaries, aerobic as well as anaerobic $NH_4^+$ sources and sinks resulted in net rates of -36 to

**Table 2. Ammonium budget for the upper Namibian and Peruvian OMZ considering aerobic and anaerobic $NH_4^+$-producing and consuming processes.** For the sake of clarity, standard errors for the individual processes determined at each station are not listed here (typically ~10% of the measured rate). Directly measured rates are in italics, the remainder were inferred from idealized stoichiometries (see S1 File for further details). Liberation of $NH_4^+$ from organic matter via oxic as well as $NO_3^-$ respiration accounts for bacterial N-uptake assuming a growth efficiency of 0.15 [66] and a C/N ratio of 6.6 for the heterotrophic community [67,68].

| | Namibian OMZ | | Peruvian OMZ | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Station** | 243 | 252 | 805 | 807 | 811 | 3[4] | 5 | 13 | 36 |
| **Site characteristics** | | | | | | | | | |
| Water depth (m) | 103 | 111 | 999 | 115 | 145 | 4,697 | 4,525 | 356 | 2,845 |
| Depth sampled (m) | 80 | 76 | 62 | 15 | 54 | 52 | 75 | 38 | 90 |
| $O_2$ (µmol $l^{-1}$) | 7.59 | 1.11 | 7.46 | ~20 | 4.16 | 4.01 | 2.60 | 3.40 | 1.49 |
| $NH_4^+$ (µmol $l^{-1}$) | 0.00 | 0.12 | 0.27 | 0.58 | 0.05 | 1.27 | 0.07 | 0.10 | 0.05 |
| **Aerobic N cycling (nmol N $l^{-1}$ $d^{-1}$)** | | | | | | | | | |
| *$NH_3$ oxidation*[1] | 21 | 93 | 89 | 49 | 13 | 60 | 5.8 | 14 | 35 |
| *$NO_2^-$ oxidation*[1] | 74 | 112 | 38 | 928 | 70 | 35 | 32 | 29 | 186 |
| **$O_2$ consumption (nmol $O_2$ $l^{-1}$ $d^{-1}$)** | | | | | | | | | |
| *Total oxic respiration* | 230 | 450 | 541 | 3,136 | 605 | 1,195 | 730 | 990 | 1,060 |
| Heterotrophic oxic respiration[2] | 161 | 254 | 389 | 2,599 | 552 | 1087 | 706 | 954 | 915 |
| **Anaerobic N cycling (nmol N $l^{-1}$ $d^{-1}$)** | | | | | | | | | |
| *$NO_3^-$ reduction*[1] | 17 | 370 | 18 | 1,010 | 0.0 | 40 | 0.0 | 0.0 | 42 |
| *DNRA*[1] | 0.0 | 12 | 0.3 | 1.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.8 |
| *Anammox*[1] | 25 | 42 | 0.0 | 112 | 9.6 | 1.6 | 4.0 | 3.6 | 2.3 |
| **$NH_4^+$ sinks (nmol $NH_4^+$ $l^{-1}$ $d^{-1}$)** | | | | | | | | | |
| *$NH_3$ oxidation* | -21 | -93 | -89 | -49 | -13 | -60 | -5.8 | -14 | -35 |
| *Anammox* | -13 | -21 | 0.0 | -56 | -4.8 | -0.8 | -2.0 | -1.8 | -1.2 |
| **$NH_4^+$ sources (nmol $NH_4^+$ $l^{-1}$ $d^{-1}$)** | | | | | | | | | |
| Heterotrophic oxic respiration[3] | 21 | 33 | 50 | 333 | 71 | 139 | 91 | 122 | 117 |
| $NO_3^-$ reduction[3] | 1.1 | 24 | 1.1 | 65 | 0 | 2.5 | 0 | 0 | 2.7 |
| DNRA[3] | 0 | 17 | 0.5 | 1.6 | 0 | 0 | 0 | 0 | 0.9 |
| **Net $NH_4^+$ rate (nmol $l^{-1}$ $d^{-1}$)** | -12 (±11) | -45 (±30) | -37 (±20) | 295 (±76) | 53 (±22) | 81 (±20) | 83 (±11) | 107 (±22) | 85 (±22) |

[1] From references [14,24,25].

[2] Heterotrophic oxic respiration = Total oxic respiration– 1.5 * $NH_3$ oxidation– 0.5 * $NO_2^-$ oxidation.

[3] Heterotrophic oxic respiration: $O_2/NH_4^+$ = 106/16; $NO_3^-$ reduction: $NO_3^-/NH_4^+$ = 212/16; DNRA: $NO_3^-/NH_4^+$ = 53/69.

[4] Station sampled for metagenomic analysis (Fig 2).

doi:10.1371/journal.pone.0133526.t002

365 nmol $NH_4^+$ $l^{-1}$ $d^{-1}$ (Table 2). Aerobic organic matter respiration could on average account for 91% of the $NH_4^+$ production.

Obviously, estimates of $NH_4^+$ liberation from organic matter are highly sensitive to changes in the C/N ratio. Considering non-Redfieldian C/N ratios of 5.3 and 10.6, as observed for surface particulate matter in the Peruvian OMZ [45], results in an increase of 55% and a decrease of 61%, respectively, in aerobic $NH_4^+$ release (assuming a constant $O_2$/C ratio). Regardless of the C/N scenario chosen, sufficient $NH_4^+$ is provided to fuel 90–100% of the combined demands of aerobic and anaerobic $NH_4^+$ oxidation at five out of six stations in the Peruvian OMZ. More negative $NH_4^+$ balances are observed for the shallow Namibian shelf OMZ. Here, sedimentary $NH_4^+$ release likely plays a more important role in driving N-loss via anammox (Fig 1c).

## Conclusions

In summary, extensive rate measurements combined with metagenomic as well as metatranscriptomic analyses show widespread potential for microaerobic respiration in the Namibian and South Pacific OMZs. Microorganisms inhabiting the OMZ use high-affinity respiratory oxidases to exploit traces amounts of $O_2$, brought in via intrusions of oxygenated surface waters, lateral advection of $O_2$-bearing water masses, or produced locally by low-light adapted phytoplankton. At the upper OMZ boundary, where micromolar $O_2$ concentrations persist, microaerobic respiration is the major mode of organic matter degradation and primary source of $NH_4^+$ for aerobic $NH_4^+$ oxidation and N-loss via anammox. The close spatial coupling of aerobic and anaerobic pathways in ($O_2$-carrying) OMZ waters is likely facilitated by formation of $O_2$-reduced microniches in sinking aggregates.

In current biogeochemical models, remineralization of organic matter exported to the OMZs is largely coupled to denitrification, typically resulting in overestimated N-loss from tropical and subtropical upwelling systems [22,69]. Denitrification by facultative anaerobic heterotrophs, however, only occurs under (near) anoxic conditions [63], and a large fraction of sinking organic matter is remineralized in more oxic upper OMZ waters. Considering aerobic microbial respiration as a major mode of remineralization of export production in this zone might help to improve model-based assessments of the current oceanic N-balance [37], as well as the effects of globally expanding OMZs and changing productivities on the ocean's future N-budget.

## Supporting Information

**S1 File.**
(PDF)

**S1 Fig. Physicochemical zonation and rates of microbial respiration in the OMZs off Namibia and Peru.**
(PDF)

**S2 Fig. Particle size distributions in the South Pacific OMZ.**
(PDF)

**S1 Table. Oxygen consumption rates in the OMZs off Namibia and Peru.**
(PDF)

**S2 Table. $O_2$ sensitivity assays in the OMZs off Namibia and Peru.**
(PDF)

**S3 Table. Abundance and taxonomic assignment of BLAST hits for low-affinity and high-affinity (bd and $cbb_3$) cytochrome oxidases of metagenomes and metatranscriptomes from the South Pacific OMZ.**
(PDF)

## Acknowledgments

We wish to thank the governments of Namibia and Peru for access to their territorial waters. Our sincere thanks go to the cruise leaders Kay Emeis (M76-2) and Martin Frank (M77-3) as well as the crews of the cruises on board R/V Meteor for their support at sea. We are grateful for the technical and analytical assistance of Gabriele Klockgether, Daniela Franzke, Violeta Leon, Linda Sin, Jasmin Franz, Pelin Yilmaz, Nicole Pinnow, Sergio Contreras, Aurelien Paulmier, Andreas Ellrott and Volker Meyer. Frank Stewart and Ed DeLong are particularly

thanked for providing metagenome and metatranscriptome sequence data from the Chilean OMZ. We thank Lars Stemmann for providing the UVP5.

## Author Contributions

Conceived and designed the experiments: TK GL MMJ CL HS HH RK JLR RAS MMMK. Performed the experiments: TK GL MMJ CL HS HH RK. Analyzed the data: TK GL MMJ HS DKD RK MH. Contributed reagents/materials/analysis tools: NPR MIG. Wrote the paper: TK GL NPR RK MH MMMK.

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